Characterization of Roasted Coffee by S-HSGC and HPLC-UV and Principal Component Analysis

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A method that differentiates among different coffees is described. Characterization is by applying principal component analysis (PCA) to the chromatographic results, obtained either by static headspace capillary GC (S-HSGC) of the roasted ground coffee or by HPLC-UV analysis of Mocha coffee extracts, or to a combination of the results of both. The possibilities of this method are illustrated by some examples concerning the differentiation of coffee samples of different origins or submitted to different roasting treatments or in mixtures of different composition.

INTRODUCTION

A variety or a blend of coffee is generally evaluated by several parameters (humidity, bean size, color, caffeine content, and so on) and through organoleptic tests by a panel of experienced tasters.

The concept of quality of a coffee has been defined in several sometimes controversial ways: one of the most general defines "the quality of coffee in the accepted sense of the term as including chemical and organoleptic properties mainly sought after by the consumer" (Pochet, 1990). From this it follows that tasting is of prime importance in the overall evaluation, although it is sometimes considered insufficiently objective. Chemical analyses are not commonly used in the overall evaluation of coffee quality, probably because too many chemical components must be considered in order for flavor and taste to be assessed as completely as is possible by tasting. The study of the chemical profile of one or more coffee fractions [e.g. headspace (HS), hot-water extract, phenolic fraction, nitrogenous fraction, etc.] can give an unbiased characterization of a coffee. However, these profiles are so complex that multivariate statistics is needed to obtain a definitive differentiation among different coffees. The volatile fraction, in particular HS, is one of the most commonly used (Merrit et al., 1969; Kallio et al., 1990; Leino et al., 1992). The use of gas chromatographic profiles combined with statistical methods to evaluate coffee was first investigated by Powers and Keith (1968) and Biggers et al. (1969): several other authors later used this approach, including Ott and Liardon (1981), Liardon and Ott (1984), Liardon et al. (1984), Liardon and Spadone (1985), Wada et al. (1987, 1989a,b), and Elmore and Nursten (1990).

The HS profile is not always sufficient to define coffee quality, in particular not as accurately as by tasting; this is probably because the HS components mainly characterize aroma (Dravnieks and O'Donnell, 1971). When HS is insufficient for an unbiased evaluation, a different fraction must be investigated as an alternative to, or combined with, HS. In the authors' experience, HPLC-UV analysis of Mocha extract gives significant profiles and is easy and quick to do and to standardize.

Principal component analysis (PCA) was chosen to analyze multivariate data, since it is successful in investigating relationships among larger numbers of variables. PCA is a useful technique for reducing the numbers of variables in a data set by finding linear combination of those variables that explain most of the variability (Wold, 1987; *Statgraphics Manual*).

This article describes a method to discriminate among samples of coffees of different origins or which have undergone different technological processes, or coffee blends of different composition. They are differentiated by applying PCA to the chromatographic results: from static headspace capillary GC (S-HSGC) analysis on roasted ground coffee or from HPLC-UV analysis on Mocha extract or to a combination of both results.

EXPERIMENTAL PROCEDURES

Coffee Samples. Samples of roasted coffees of different origins, of coffee submitted to different technological treatments, and of different blends of coffees were supplied by Lavazza SpA, Torino, Italy. The original green coffees with parchment were stored in the areas of production. Ten samples (50 g) of each coffee variety or blend were hermetically sealed under vacuum in nonpermeable polypropylene/aluminum/polyethylene packages and stored at −20 °C after roasting, until used for chemical analysis. More details about the samples analyzed will be reported under Results and Discussion.

Reference Standards. Pure reference standards of chlorogenic acid, trigonelline, and nicotinic acid were obtained from Fluka Chemika (Buchs, Switzerland).

S-HSGC Analysis. A Carlo Erba HS-250 automatic HS injection system assembled on a Carlo Erba 4160 GC unit was used.

Sample Preparation. Each coffee package was left for 30 min to reach room temperature. At least 10 2-g samples of each coffee were hermetically sealed in 10-mL vials and again stored at −20 °C. Each vial was again left for 30 min to reach room temperature before analysis and then equilibrated for 3 h in the thermostatic bath of the HS injector at 70 °C before injection.

CGC Analysis. One milliliter of the vapor phase is directly injected in the GC system. An FSOT-high temperture silylated OV-1 column (df = 3 μm, i.d. = 0.32 mm, length 25 m) was used. Chromatographic conditions: injection system, splitless; time, 40 s; injector temperature, 230 °C; temperature program, from 20 °C (5 min) to 130 °C at 3 °C/min; detector, FID; temperature, 250 °C; carrier gas, hydrogen; flow rate 2 mL/min.

GC/MS Analysis. GC/EI-MS analysis were carried out on a Hewlett-Packard 5988 A GC/MS system provided with a Hewlett-Packard 5890 GC unit. Capillary GC separations were carried out with the same column and under conditions analogous to those reported in the previous paragraph. Carrier gas was helium.

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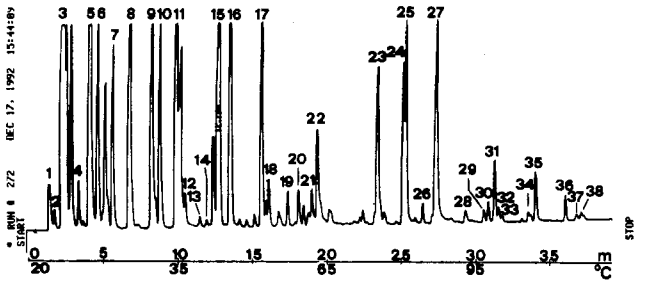

Figure 1. S-HSGC profile of an Arabica coffee of Colombian origin.

Table I. Components Identified in a Colombian Arabica Coffee HS Fraction

peak	MW	name	peak	MW	name
1	44	ethanal	21	81	1-methylpyrrole
2	48	methyl mercaptan	22	79	pyridine
3	58	propanal	23	100	2-methyltetrahydro-furan-3-one
4	60	methyl formate			
5	58	acetone	24	94	2-methylpyrazine
6	68	furan	25	96	2-furfuraldehyde
7	74	methyl acetate	26	108	cresol
8	72	isobutanal	27	98	furfuryl alcohol
9	86	diacetyl	28	86	butyrolactone
10	72	2-butanone	29	126	furfuryl formate
11	82	2-methylfuran	30	110	2-acetylfuran
12	60	acetic acid	31	108	2,6-dimethylpyrazine
13	74	acetol	32	108	ethylpyrazine
14	94	dimethyl disulfide	33	108	2,3-dimethylpyrazine
15	86	3-methylbutanal	34	114	furfuryl mercaptan
16	86	2-methylbutanal	35	110	5-methyl-2-furfur-aldehyde
17	100	2,3-pentanedione			
18	88	acetoin	36	140	furfuryl acetate
19	96	2,5-dimethylfuran	37	122	methylethylpyrazine
20	80	pyrazine	38	122	trimethylpyrazine

Mocha Extract Preparation and HPLC-UV Analysis. Coffee brews were prepared in a commercially available Mocha coffee pot (Carmencita, Mocha Italian type, stainless steel, Lavazza, Torino, Italy) under strictly standardized conditions. An amount of 17 g of coffee was extracted with 185 mL of water for an extraction time of 5 min. The brews were filtered on a Millipore Millex LCR (0.5 μm) (Millipore Waters, Milford, MA) and then directly submitted to HPLC-UV analysis.

HPLC-UV Analysis. The HPLC-UV analyses were carried out following the method proposed by Van der Stegen and Van Duijn (1980) and modified in the authors's laboratory. HPLC-UV was carried out on an HPLC system consisting of two Model 501 Waters Associates LC pumping units, an automated gradient controller (Waters Associates), a Model 7010 Rheodyne valve loop injector fitted with a 20-μL loop, and a Waters Associates Model Lambda-Max 481 LC spectrophotometer with UV detector. A LiChrosorb RP-18 (10 μm), 4.6 mm i.d. × 250 mm, (Merck, Darmstadt), was used. UV detection was carried out at two different wavelengths, 325 and 272 nm. The following elution program at room temperature (25 °C) was used: 15% methanol in a potassium citrate solution (pH 6) to 40.5% methanol in 25 min of linear gradient.

Data Elaboration. The chromatographic data both from HS-CGC and HPLC-UV were processed on HP 3396A computing integrators and then transferred on-line to an HP Vectra 386SX personal computer (Hewlett-Packard, Grenoble, France) where they were elaborated through a PCA program (Statgraphics, Statistical Graphics Corp., Rockville, MD). A homemade program was used to convert the file format from computing integrators into a form compatible with the statistics program.

RESULTS AND DISCUSSION

One of the aims of this study was to develop a method which was quick, easy to automate, provided an objective evaluation of roasted coffee, and was complementary to tasting. Therefore preference was paid to sample preparation methods, i.e., S-HSGC and Mocha extract, with no intermediate steps before the chromatographic analyses. Although enriched HSGC techniques are more widely used, S-HSGC was preferred in this case, because it is easier to standardize and requires less sample handling.

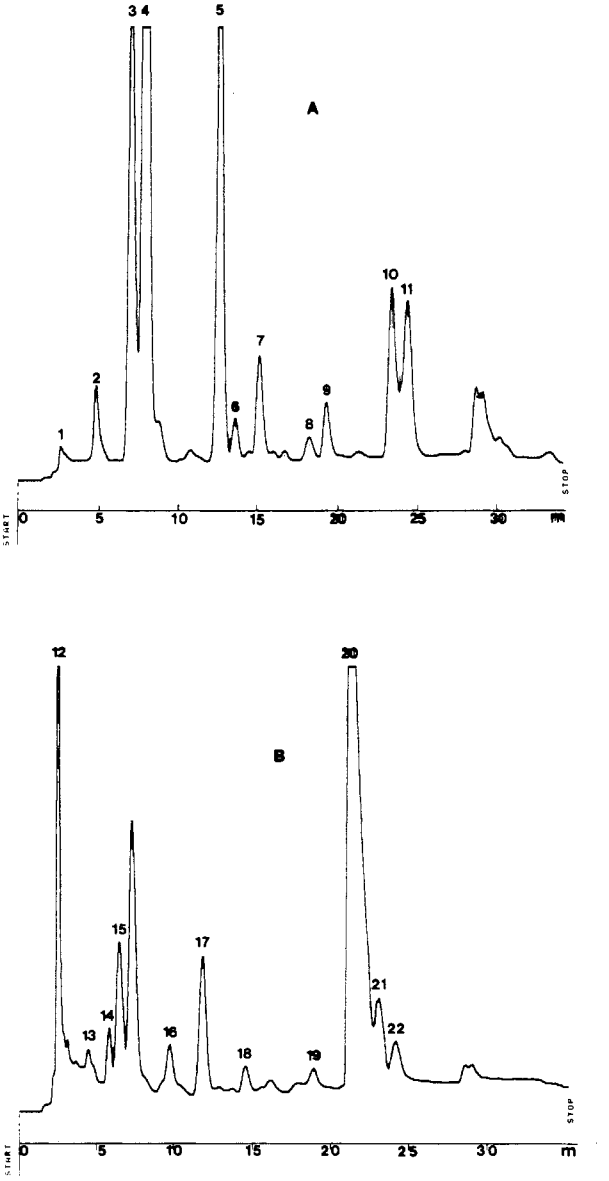

Figure 2. HPLC-UV patterns of the Mocha extract of an Arabica coffee of Colombian origin carried out at 325 (A) and 272 nm (B).

Figure 1 reports the chromatographic profile of S-HSGC of an Arabica coffee of Colombian origin. Table I reports the components identified by S-HSGC-MS in the HS in question. The results of a study concerning HS composition will be the ojbect of a forthcoming publication (Bicchi et al., unpublished results).

Figure 2 reports the HPLC-UV patterns of the Mocha extract of the same coffee sample carried out at both 272 and 325 nm.

The use of two detection UV wavelengths improved recognition of the coffee extract components: in particular chlorogenic acids are better detected at 325 nm, while purine alkaloids and trigonelline are better detected at 272 nm.

Three examples illustrate the effectiveness of the statistical methods applied to the chromatographic results.

The first concerns the discrimination of some Arabica coffee samples from four different geographical areas

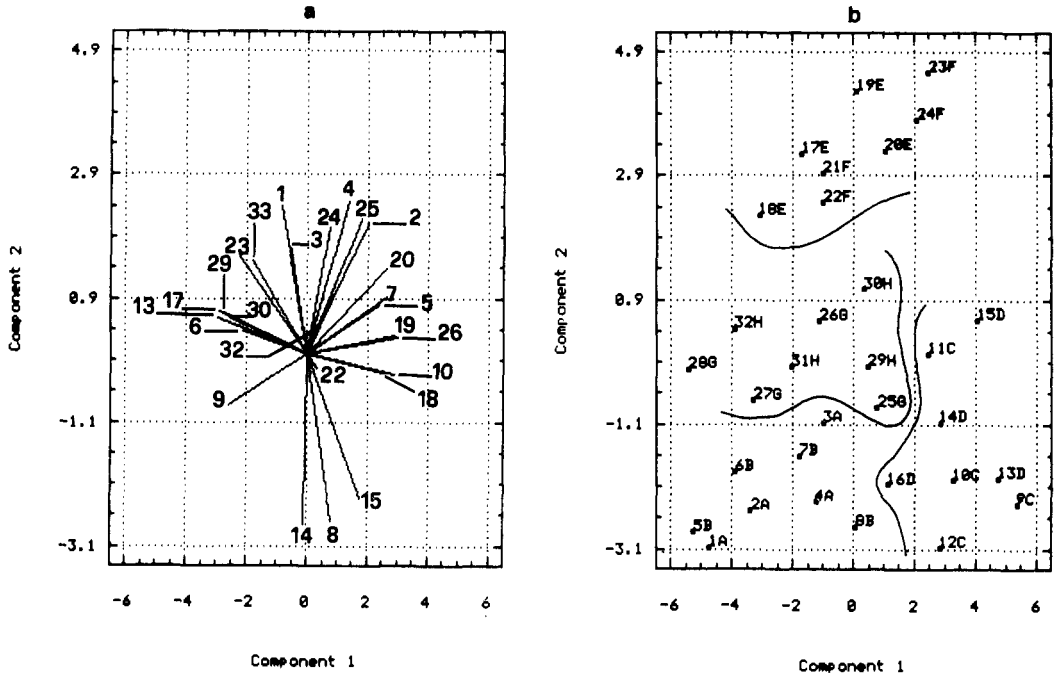

Figure 3. (a) Distribution of the S-HSGC loadings for PCA of Arabica coffees from Costa Rica, Guatemala, Honduras, and Nicaragua. (b) PCA scatterplot of the same coffee samples; PC1 = 52.859%, PC2 = 32.381%. For clarity the different areas of origin have been outlined by hand.

Table II. Origin and Harvesting Time of the Investigated Coffees[a]

sample no.	origin	harvesting time	sample no.	origin	harvesting time
1A	Costa Rica	12/91	17E	Guatemala	12/91
2A	Costa Rica	1/92	18E	Guatemala	1/92
3A	Costa Rica	12/92	19E	Guatemala	12/92
4A	Costa Rica	1/93	20E	Guatemala	1/93
5B	Costa Rica	12/91	21F	Guatemala	12/91
6B	Costa Rica	1/92	22F	Guatemala	1/92
7B	Costa Rica	12/92	23F	Guatemala	12/92
8B	Costa Rica	1/93	24F	Guatemala	1/93
9C	Honduras	12/91	25G	Nicaragua	12/91
10C	Honduras	1/92	26G	Nicaragua	1/92
11C	Honduras	12/92	27G	Nicaragua	12/92
12C	Honduras	1/93	28G	Nicaragua	1/93
13D	Honduras	12/91	29H	Nicaragua	12/91
14D	Honduras	1/92	30H	Nicaragua	1/92
15D	Honduras	12/92	31H	Nicaragua	12/92
16D	Honduras	1/93	32H	Nicaragua	1/93

[a] The letters A–H indicate different areas within each country.

(Costa Rica, Guatemala, Honduras, and Nicaragua). Eight different samples from each of these geographical areas were investigated; Table II reports their characteristics. Each sample was analyzed six times by S-HSGC. The HS profiles were submitted to a detailed investigation by PCA. Routine statistical analyses were carried out on the 26 peaks which showed detectable and reproducibly measurable areas in all the samples under investigation. PCA was applied to the mean areas, calculated over six analyses, of each peak from each sample, so as to obtain a clearer scatterplot. Figure 3a reports the distribution of the loadings considered for PCA and Figure 3b the scatterplot of principal components of the coffees in question, which clearly distinguishes the origins of the samples. For clarity the different areas of origin have been outlined by hand.

The second example concerns the distinction of six different coffees, respectively, of pure Arabica (a), pure Robusta (b), and four mixtures of the two in the following ratios: 50% Arabica–50% Robusta (c), 40% Arabica–60% Robusta (d), 20% Arabica–80% Robusta (e), 20% Arabica–80% Robusta (f). Samples e and f were prepared with

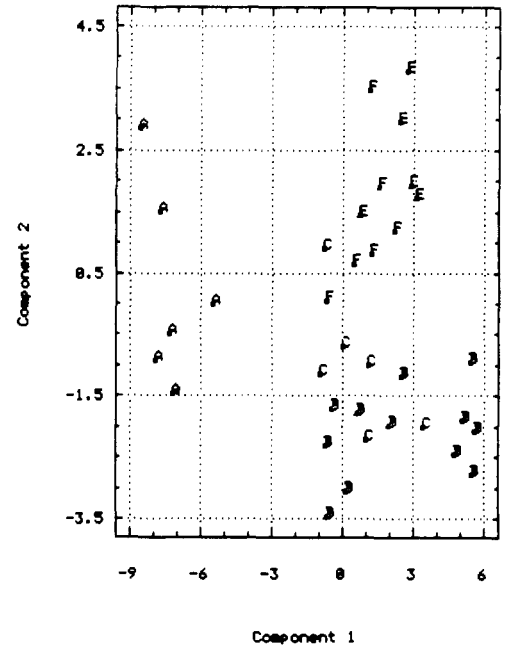

Figure 4. PCA of the S-HSGC profiles of Arabica/Robusta coffee blends. PC1 = 65.213%, PC2 = 21.499%.

two Robustas of different origins. Figure 4 reports the PCA of the S-HSGC profiles. With S-HSGC/PCA analysis, samples a and b were clearly distinguished, while samples c–f were not unambiguously discriminated.

HPLC-UV profiles at both 272 and 325 nm of Mocha extracts were then submitted to PCA. Routine statistical analyses were carried out on the 22 peaks which showed detectable and reproducibly measurable areas in all the samples under investigation. Figure 5a reports the distribution of the loadings considered for PCA of the coffees under investigation. Figure 5b reports the HPLC-UV (272 + 325 nm) PCA scatterplot of the same coffees samples. HPLC-UV at 272 and 325 nm of the Mocha extracts discriminated better among the coffees a, b, and d but discrimination among c, e, and f was poorer than by

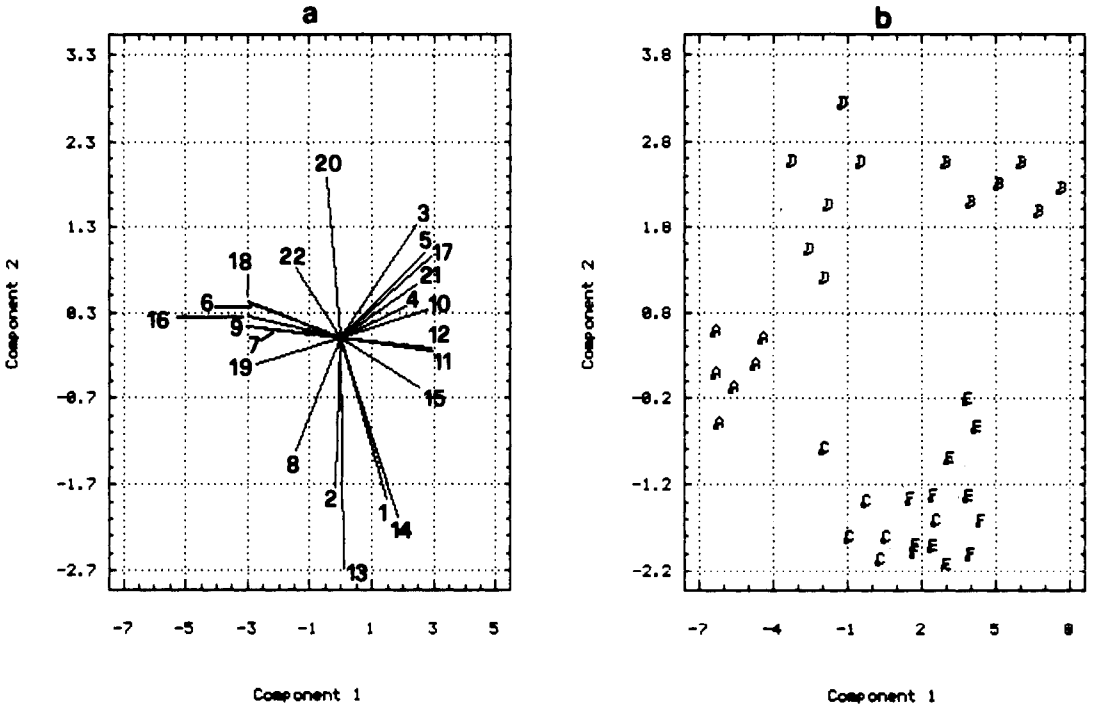

Figure 5. (a) Distribution of HPLC/UV (272 + 325 nm) loadings for PCA of Arabica/Robusta coffee blends. (b) HPLC-UV (272 + 325 nm) PCA scatterplot of the same coffee samples; PC1 = 63.806%, PC2 = 19.684%.

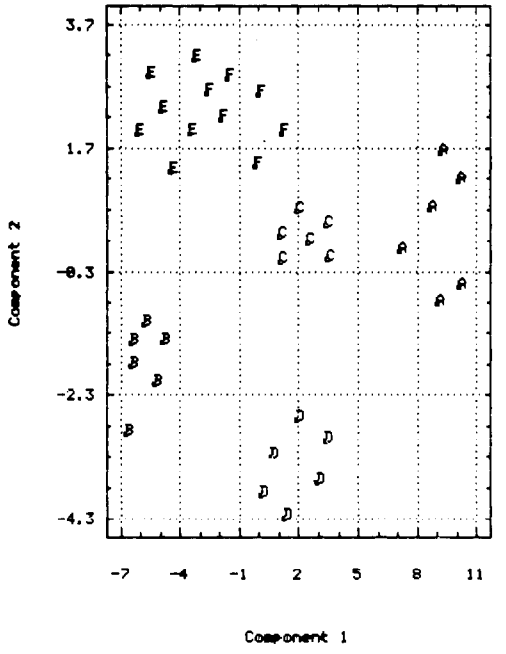

Figure 6. Combined S-HSGC/HPLC-UV PCA scatterplot of Arabica/Robusta coffee blends: PC1 = 66.140%, PC2 = 14.463%.

S-HSGC. Figure 6 reports the PCA scatterplot of the combination between S-HSGC and HPLC-UV (272 + 325 nm) variables. The combination of the S-HSGC results, together with those of HPLC-UV of the Mocha extracts of the same samples, afforded a complete and unambiguous discrimination.

The third example concerns the characterization of five samples of the same Arabica coffee submitted to different roasting programs, with roasting temperatures and times increasing from sample 1 to sample 5. The taste of the Mocha extract of the five coffee samples in question was evaluated by a panel of 20 tasters who tasted each coffee sample under the same conditions; the resulting descriptive profiles are in Figure 7 (Gillette, 1990). Coffees were

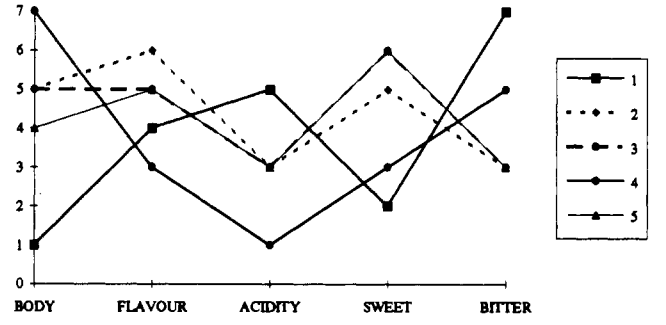

Figure 7. Descriptive analysis of five Arabica coffee samples submitted to different roasting programs: 1 = very low; 2 = low; 3 = medium low; 4 = medium; 5 = medium high; 6 = high; 7 = very high.

evaluated at room temperature and without adding sugar. The descriptive profiles clearly show that sample 1 is very different from 4 and that both are different from the groups 2, 3, and 5, which were classified as being very similar.

PCA was applied to the mean areas, calculated over six analyses, of each peak from each separate sample, so as to obtain clearer scatterplots. Figure 8a reports the scatterplot of PCA applied to S-HSGC profiles. The results of PCA, applied to S-HSGC profiles, were not in agreement with the description profiles. On the contrary, PCA applied to HPLC-UV (both at 272 nm and at 325 nm) profiles of Mocha extracts gave a coffee distribution in good agreement with the description profiles, together with a clearer discrimination. Figure 8b reports the scatterplot of PCA applied to HPLC-UV (272 + 325 nm) profiles.

CONCLUSIONS

S-HSGC of the roasted ground coffee and HPLC-UV analysis of Mocha coffee extracts in combination with PCA were confirmed to be complementary for an objective discrimination of coffee samples. The use of the two profiles combined generally affords a better agreement with tasting, in addition to discrimination. At present

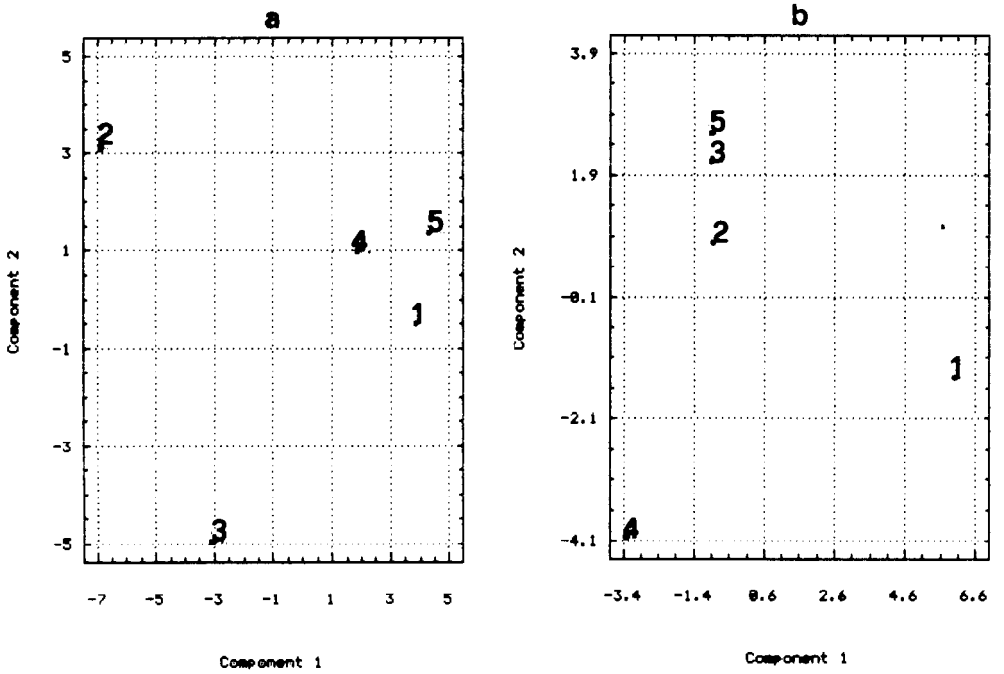

Figure 8. (a) S-HSGC PCA scatterplot of PCA applied to a washed Arabica coffee sample submitted to different roasting programs: PC1 = 58.684%, PC2 = 24.272%. (b) HPLC-UV (272 + 325 nm) PCA scatterplot applied to the same samples: PC1 = 55.426%, PC2 = 33.507%.

there is no evidence about which of the two profiles gives the greatest contribution to coffee evaluation: further studies on this subject are under way.

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